**Adaptive Immunity**

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These notes are meant for your personal education and to help with this and other courses you may have on microbiology. I will not test you on history but for those of you who want to pursue a career in biology and especially in immunology you may find these references a source of some inspiration.

**Outline – historical perspective on the discovery of Adaptive Immunity**

**The origins of adaptive immunity**

A brief overview of major historical observations highlighting the existence of acquired immunity

**430B.C. Plague of Athens (Thucydides)**

* Survivors are resistant to the subsequent infections
* First documented evidence of anamnestic immunity (memory)

**1796A.D. Smallpox in Europe**

* Edward Jenner observes that milkmaids exposed to cowpox are resistant to small pox
* Experimentally demonstrates that infection with cowpox is protective to subsequent infections

**1870-1890 Louis Pasteur and Robert Koch**

*(good read for this: ‘The Microbe Hunters” https://laurieximenez.files.wordpress.com/2016/03/2-microbe-hunters-paul-de-kruif.pdf)*

* Rivalry for dominance in European microbiology – leads to the development of vaccines for: anthrax, chicken cholera, rabies
* (one) Major finding is that attenuation (killing/reducing pathogenicity) of microorganisms is required for development of good vaccines

**Late-1800’s Emil von Behring and Shibasaburo Kitasato** and the discovery of serum factors as mediators of transferrable immunity

FIRST Nobel prize in Medicine (1901) to Emil von Behring for "for his work on serum therapy, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths."

- First evidence that there is a specific factor produced in the body that can protect against subsequent infections (antibodies)

*Treatment of SARS-CoV2 with convalescent serum is essentially the same old fashioned medicine described by these two over 100 years ago….*

**Discovery of Molecular and Cellular Basis of Acquired Immunity**

https://www.nobelprize.org/prizes/medicine/1980/summary/

Early 1900’s – Discovery of tissue antigens (called HLA in humans, MHC in all mammals)

* George Snell (Jackson Labs) Nobel Prize 1980 for ‘The discovery of genetically determine structures on the cell surface that regulate immunological reactions.’

Was interested in transplant rejection (in particular rejection of tumors) between mice strains

Development of congenic mouse strains (mice which are nearly genetically identical except for at a single region of the genome) allowed for understanding genetic elements controlling transplant rejection. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4530560/)

Mid-1900’s – Discovery of immunological tolerance

* 1945, Ray Owen and Cattle twin observations: Observed that non-identical twin cattle shared blood system in utero and afterwards including red blood cells bearing ‘foreign antigens’. (see a review on this below)

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5063071/>

He wrote in the discussion – ‘several interesting problems in the fields of genetics, immunology and development are suggested by these observations. Most of them are still highly speculative and will not be considered here.’

* Sirs Peter Medawar and Frank Macfarlane Burnet. Nobel Prize 1960 for ‘The Discovery of Acquired Immunological Tolerance”

Medawar – developmental exposure to foreign antigens (mouse MHC) leads to tolerance (fetus) or immunity (adult). Validated Ray Owen’s findings in mouse models in 1953 establishing an experimental strategy to induce tolerance in utero.

Burnet – Clonal selection theory

Postulates how specific antigen receptors can be encoded within a single cellular unit (lymphocytes) and passed on to their daughter cells.

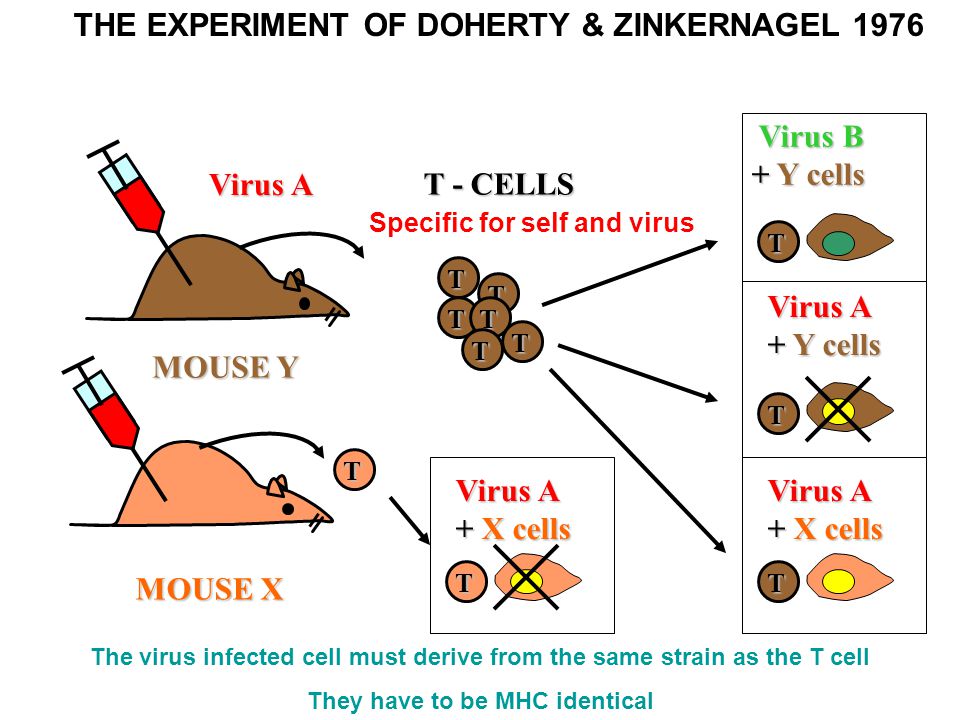
Basis for adaptive immunity for all B and T cells

The Key concepts are that lymphocytes or adaptive immune cells must first learn what is or is not the host organism, and after that these cells can be selected for and expanded to target any foreign antigens.

1970’s – Rolf Zinkernagel and Peter Doherty. Nobel Prize 1996 for “the specificity of the cell mediated immune defence”

Found that T lymphocytes recognize foreign antigens presented in the context of MHC (HLA) molecules

Establish a direct molecular mechanism for how T cells are able to search for infected cells and kill them – T cells from a mouse vaccinated against a virus will kill cells from the same mouse strain (with the same MHC) in cultures – whereas they will not kill cells from a foreign mouse strain even when they are infected with the same virus (illustration below).



**2020 – SARS-CoV2 Pandemic!**

Immunity hits the public square ☺ (from the NYtimes Oct 5 2020)

Diagram

Description automatically generated

**Cells of the Adaptive Immune System**

**LYMPHOCYTES (express antigen receptors – look for antigen on antigen presenting cells):**

**T lymphocytes ( also called T cells) (T = Thymus – most T cells are made in the thymus)**

Express rearranged T cell receptors (TCRs) and generally express CD3 proteins (CD3E, CD3D, CD3G)

Interact directly with target cells – if a cell has a virus inside of it the T cell will receive signals directly from the infected cell and act directly upon it in many cases. T cells typically respond to infections by (A) secreting cytokines locally to activate or recruit local lymphocytes or (B) direct killing of infected or damaged cells.

**Types:**

**-- Alpha/beta (>90% of CD3+ T cells)**

**The major class of T cells.** Denoted alpha/beta because the T cell receptor (TCR) of these cells is composed of a heterodimer of the TCR alpha and beta genes

**Classified into two main subgroups**

***-- CD4+ T cells*:** interact with MHC Class II presenting peptides

Termed – T helper cells (Th cells) because they secrete cytokines to ‘help’ boost immune responses

***-- CD8+ T cells*:** interact with MHC Class I presenting peptides

Termed – Cytotoxic T lymphocytes (CTL) because they are specifically involved in directly killing infected target cells

**-- Gamma/delta (typically 0-10% in blood)**

Rare cells with less clear function. These T cells rearrange a gamma and delta T cell receptor rather than alpha/beta. Otherwise they are similar. Potentially important at specific tissue barriers (skin/liver/intestine).

Can be separated in subtypes – but typically assigned to groups based on the TCR genes used (rather than CD4 or CD8). This is because they have very restricted TCR usage compared to alpha/beta cells. Can be CD3 negative as well.

**B lymphocytes (also called B cells) (B = Bursa of Fabricius – Most B cells are produced in the Bone Marrow, but B stands for an obscure bird organ)**

B cells are identified by expression of B cell receptor (BCR) components and the surface proteins CD19 and CD20.

**Types:**

* **Naive B cells**

Newly made B cells that have recombined a B Cell Receptor but have not yet been activated by ‘cognate antigen’ so do not produce secreted antibodies.

* **Memory B cells**

A naive B cell that has been activated will undergo ‘maturation’ and further rearrange the BCR so that it can potentially make antibodies. Memory B cells remain in the circulation for decades and upon reactivation give rise to Plasma Cells.

* **Plasma cells**

Plasma cells are believed to be very long-lived and reside in the bone marrow or the intestines mainly. These cells are antibody producing ‘factories’ that generate large amounts of secreted antibodies with specificity for antigens to which the host was previously exposed.

Notes: It is fairly well appreciated that a successful vaccine works by generating good memory B cells and plasma cells, rather than T cells. Nonetheless there is a bias in the literature towards study of T cells and ILCs… In the HIV vaccine field there is a large movement to avoid vaccines designed to make strong T cell responses in favor of those that make good antibody responses at present.

However, in the SARS-CoV2 pandemic there has been a growing confusion about whether B cell immunity or T cell immunity is more protective. A major debate concerns whether people with pre-existing T cell immunity to related Coronaviruses (‘cross reactive’ T cell immunity) are afforded some protection against SARS-CoV2. This could happen if the virus enters the nasal pathways or lungs but encounters memory T cells that are capable of recognizing viral proteins because they share sequence similarity with other Coronaviruses. These cells could then rapidly kill infected epithelial cells before the virus is able to replicate enough to cause a major infection. Likewise, these T cells could promote worse disease if they were to trigger an inflammatory response that somehow interfered with the innate immune system or with the development of more specific B and T cell immunity to the SARS-CoV2 viral proteins. At this point it is unclear.

**Other lymphocyte subsets (innate/adaptive overlap)**

**Innate Lymphoid Cells (ILCs)**

Several categories have been defined – basically seem to function as T cells without a T cell receptor and may be a rapid source of cytokines triggered by environmental signals – thought to be non-specific due to a lack of a TCR.

**Natural Killer Cells (NK cells)**

**-** Potentially capable of long-lived memory like other adaptive immune cells, recognize specific families of receptors and absence of MHC/HLA proteins. Tumors and viruses both have evolved the capacity to downregulate HLA molecules to avoid having their own mutant or foreign peptides from being presented to T cells. NK cells look for cells without HLA and attack them as a backup against this defense.

**MAIT cells (Mucosal Associated Innate T cells)**

Express a semi-invariant TCRa chain (meaning they tend to use a common Va subunit – Va7.2 in humans). Thought to function in liver and mucosal tissues and have specificity for microbial riboflavin precursor derivatives (such as 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil (5-OP-RU), presented by an MHC class I-related molecule (MR1).

**NK T cells (Natural Killer T cells)**

**ANTIGEN PRESENTING CELLS (Express HLA/MHC – display antigen to lymphocytes):**

**Dendritic Cells (DCs) – produced in the bone marrow or from specialized antigen presenting cell precursors that migrate to lymphoid tissues. The dominant population associated with directing adaptive immune responses.**

Dendritic cells were first identified based on their distinctive appearance – they have large dendrites making them look like a bush at a haunted house.

Immature dendritic cells are round in appearance, however after activation by ‘innate’ signals (pattern recognition receptors, toll like receptors) and antigen uptake (phagocytosis) they migrate to lymphoid structures where they extend dendrites so that they can interact with lots of T cells and present antigens.

This is why DCs are considered a ‘bridge’ between innate and adaptive immunity.

**Macrophages -** maycome from similar precursor populations as DCs, distinguished by having greater phagocytic activity, direct killing of bacteria, and don’t have elongated dendrites. Typically associated with bacterial responses.

**Plasmacytoid Dendritic Cells (pDCS) –** Lymphoid-like cells that secrete large amounts of Interferon alpha and can present antigens to T cells. Thought to be involved in viral responses primarily.

**The T cell receptor**

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**Outline**

**Two major lymphocyte subsets with antigen receptors**

**1. T cells – T Cell Receptor (TCR)**

2. B cells (next lecture – Lisa Westerberg)

**Problem: There exists in nature a nearly infinite combination of possible foreign infectious agents that are potentially lethal to a multicellular organism. How do large animals generate populations of lymphocytes that can sense all possible antigens even without prior knowledge of the antigens that exist in nature?**

* **Issues to consider**
* **Limited potential to encode diversity in genome.**

If you wanted to make a single gene for all possible T cell receptors needed to kill all foreign pathogens, you would have to have thousands if not millions of genes just for this purpose – we have about 25,000 known genes in total as of 2020.

* **Must be able to discriminate between ‘self’ and ‘non-self’**

All of your T cells with their unique T cell receptors must be able to sense and respond to all foreign organisms but NEVER to your own 25,000 proteins. This is a super complicated job.

**History/Background**

**Discovery of the Generation of Antigen Receptor Diversity**

***1st Hypothesis (Paul Erlich 1900) – adaptive immune cells have an enormous array of diverse ‘side chains’ which can see all things in nature. When they encounter something new, they fall off the cell and that cell then starts secreting large amounts of the lost ‘side chain.’***

**-** Postulated that each cell can see all things. Incompatible with limited genome encoding only 20-30,000 genes. So theoretically a good model but incorrect because it says the genome rather than the cell is the unit selected on the basis of antigen specificity.

***https://en.wikipedia.org/wiki/Side-chain\_theory***

***2nd Hypothesis (Karl Landsteiner 1920s-1940s) – antigens themselves were capable of converting some antigen recognition protein (antibodies), so that it would adapt a shape capable of recognizing the antigen. The PROTEIN is the source of antigen recognition.***

* Cannot effectively explain why secondary (anamnestic) responses are often better than the original response – also why the adaptive immune system attacks foreign antigens but not self.
* The basic thought here is that you have an antibody that has no shape, and when it sees something foreign it will conform to the shape of that thing, switching shapes as it encounters any additional foreign thing.

***3rd Hypothesis (Frank Macfarlane Burnet 1950s) – Clonal Selection Theory. The CELLS are the unit of antigen recognition. Each cell has a unique specificity and this is passed on to that cells progeny.***

* This theory was compatible with the idea of adaptive memory responses since clonal expansion means that lymphocytes that are activated will generate copies of themselves that share the same antigen recognition receptor. It also means that tolerance to self could be achieved by ‘deleting’ or ‘tolerizing’ cells that react against self-antigens during development of the organism (more later).

Burnet is a great writer – you can read his Nobel Lecture for this discovery if you are interested here:

*https://www.nobelprize.org/uploads/2018/06/burnet-lecture.pdf*

**Mechanism for Generating T Cell Receptor Diversity**

**The Structure of the TCR genomic Locus**

**Two separate alleles/genomic regions (for alpha/beta T cells)**

**(2 alleles for both! Meaning 2 chances to generate a TCR a or b chain)**

**TCR alpha chain (human): Chromosome 14: 21,500,000-22,600,000bp**

Contains 2 types of gene segments (# = specific number of a single segment)

**T cell receptor V alpha segments (TRAV#):** approximately 70

**T cell receptor J alpha segments (TRAJ#):** 61

**TCR beta chain (human): Chromosome 7: 142,200,000-142,900,000bp**

**Contains 3 types of gene segments**

**T cell receptor V beta segments (TRBV#):** 52

**T cell receptor D beta segments (TRBV#):** 2

**T cell receptor J beta segments (TRBJ#):** 13

***V-segments:*** Variable segments

***D-segments*** Diversity segments

***J-segments:*** Joining segments

**C-Segments**: Constant regions - structural

**Basic Concept:** During T cell development, genomic rearrangements occur at both of these genomic regions to bring a V segment in contact with a J-segment (for TCR alpha) or in contact with a single D-segment, which is then joined to a single J-segment (for TCR beta).

**How Does TCR Rearrangement Occur?**

**-- Both B and T cells use similar molecular pathway to generate diverse antigen receptors**

**(Background reading: G.O.D.s Holy Grail: Discovery of the RAG proteins:** DOI: https://doi.org/10.4049/jimmunol.180.1.3**)**

**Important molecules for T cell receptor generation**

**1. RAG genes (RAG1 and RAG2) – Recombination Activating Genes**

**2. Terminal deoxynucleotidyl transferase (TdT)**

**Video overview of VDJ: https://www.youtube.com/watch?v=QTOBSFJWogE**

Video is for Immunoglobulin (BCR/antibody) but the mechanisms are similar and names of segments are the same for TCR and BCR

***Susumu Tonegawa (1970s):*** Nobel Prize for Medicine 1987 ‘The discovery of the genetic principle for generation of antibody diversity’

* Asked “Does the germline DNA sequence which contains genetic elements associated with antigen receptors change over time?”

Germline DNA in embryos corresponding to regions where antibody genes were theorized to exist underwent ‘reshuffling’ during development so that lymphocytes had rearranged the order of the genes.

***David Baltimore (1974):*** Wrote a commentary in Nature speculating that terminal deoxynucleotidyl transferase (TdT), which was found specifically in thymus cells of multiple species, might be a lymphocyte antigen receptor diversity gene.

- TdT adds deoxyribonucleotides to the ends of DNA - later identified as being expressed during T cell development and adds random nucleotides to the V-D-J joined regions as the DNA is reconnected. This increases potential diversity by over 1 million-fold

***David Schatz and David Baltimore (1988):*** Since only lymphocytes (B cells in this case) rearrange the antigen receptor locus – can transferring genes expressed by B cells into fibroblasts cause rearrangement in fibroblasts? – YES

* First discovery of a single gene causing recombination (RAG)

**Basic Premise for TCR Rearrangement:**

First RAG-1 and RAG-2 cooperate to cause double stranded DNA breaks at target regions in the TCR locus.

These regions are then joined together in a ‘random’ fashion to generate potential T cell receptor genes.

TdT adds random nucleotides to the junctions of these joined regions to increase diversity (only happens after birth – not during development – not necessary for TCR formation, but important to increase diversity)

The re-arranged receptor is transcribed to make the protein – if the rearrangement causes ‘out of frame’ messenger RNA, no protein is made and the cell re-arranges the other allele. If both fail the cell dies due to lack of survival signals (required for survival).

(More on T cell Selection – TCR selection – in coming lecture on thymus and T cell development)

**Important Details to Understand About TCR Structure**

**Several Components of TCR Important for Signaling**

**Vb-Db-Jb // Va-Ja = TCR antigen sensing region**

* This portion of the TCR heterodimer interacts with MHC and peptide to ‘scan’ cells for the presence of foreign proteins

**CD4/CD8 = co-receptor**

* Determines if T cells interact with MHC Class II (CD4) or Class I (CD8)
  + CD4 is the main receptor for HIV virus

**CD3 (complex with multiple proteins – CD3, CD3, CD3 and CD3 chains)**

* Critical for T cell receptor signaling after recognizing ‘cognate’ antigen. (cognate antigen = peptide that a TCR is specific for)

**Basics on T cell Signaling**

When a T cell encounters an antigen presenting cell (APC) which is presenting a cognate antigen, the TCR complexes on the T cell surface cluster into a ‘cSMAC’ or central supramolecular cluster.

A ‘pSMAC’ or peripheral supramolecular cluster is also formed from adhesion molecules – this stabilizes the signaling ***synapse***.

This clustering leads to the generation of an intracellular signaling complex that causes T cell activation.

This is a lecture from the world leader on T cell synapse signaling (Michael Dustin) – he lectures on ALL aspects of T cell immunity so this is a great review of our lectures! :

https://www.youtube.com/watch?v=b-kzPVdfTCM

**THYMUS SELECTION (I and II)**

**Major Concepts:**

**1. The thymus is the organ where most (if not all) T cells are produced**

Most active from 10 gestational weeks fetal development until 10-20 years of life.

Undergoes ‘involution’ in adulthood and new T cell production shifts to primarily naive T cell division in the periphery

- **Thing to consider** – what is a main difference between a new T cell made in the thymus and a new T cell made in the periphery by naive T cell divisions?

**2. Hematopoietic progenitor/stem cells enter the thymus from the blood where the multiply and give rise to T cell progenitors. These T cell progenitors eventually make mature ‘Naive’ T cells.**

**Mature =** Express a Rearranged functional T cell receptor (composed of alpha and beta (or gamma and delta) TCR chains.

**Naive =** Have not been activated yet by interactions with antigen presenting cells expressing a cognate antigen

I just use mature to indicate that the T cell is no longer a progenitor – when a naive T cell is activated you call it an ‘effector’ or a ‘memory’ T cell. One could also call it ‘antigen experienced.’

**3. There is a well-defined pathway to generate a naive T cell from a progenitor – you should know the basic stages.**

**Order of Development:**

**(In the bone marrow)**

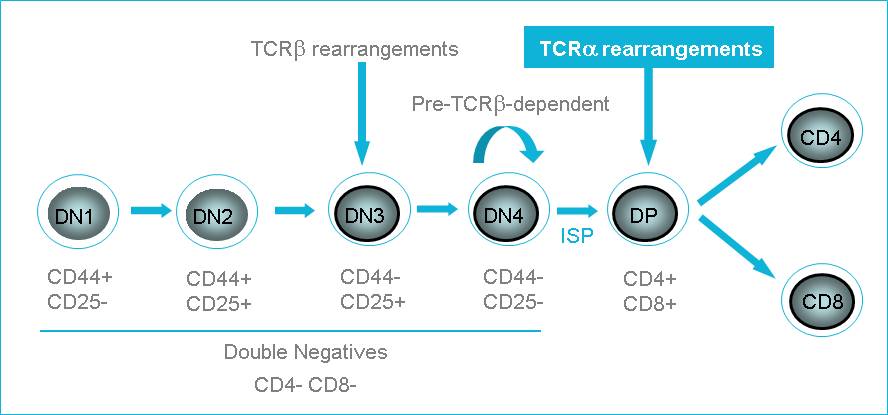
Hematopoietic Stem Cell 🡪 Common lymphoid progenitor (CLP)

**(In the Thymus)**

CLP 🡪 Double Negative Thymocyte (DN1-DN4) 🡪 Double Positive Thymocyte (DP) 🡪 Single Positive Thymocyte (SP4 or SP8 depending on expression of CD4 or CD8) 🡪 Mature (naive) CD4+ or CD8+ T cells exported to blood.

There are 4 stages of DN thymocyte that have been described based on marker expression – A T cell progenitor is fully committed to be a T cell at DN2-DN3 stage and beta chain rearrangement occurs at DN3-DN4 stage

Note in the diagram below: ISP (immature SP) is a step where cells may temporarily become SP before going back to a DP state – the actual steps of how this process works (and why) are still not fully determined.



**4. The thymus is divided into two regions that are visible to the naked eye on sections – These regions serve specific functions in T cell development (described more in coming sections)**

**1) The Thymic Cortex (cortical zone)**

This is a densely packed region containing many early stage developing T cells (mostly DN and DP)

**2) The Thymic Medulla (medullary zone)**

This region is less packed with cells and appears lighter in tissue sections. It contains more mature T cell progenitors and fully matured T cells

**5. There are some cell types in the thymus that are important to remember.**

**A) Thymocytes (refers to all stages of T cells developing in the thymus including mature T cells – called mature thymocytes)**

When a thymocyte leaves the thymus it is then called a ‘naive’ T cell

Thymocytes are found in both the cortex and medulla

**B) Cortical Epithelial Cells (cTECs)**

Main function is to instruct ‘positive selection’ – express special forms of proteolytic machinery to ensure efficient constant loading of MHC Class I and MHC Class II with peptides for constant high exposure of SELF-MHC to developing DP thymocytes

**C) Medullary Epithelial Cells (mTECs)**

Mediate ‘negative selection’ – they express a unique transcription factor called AIRE (autoimmune regulator). This transcription factor can activate the expression of many genes for MHC presentation that are tissue-restricted and thus would not be normally expressed for thymocytes to see in the thymus.

**D) Thymic Dendritic Cells/Macrophages (0.5% of cells)**

May play a role in presenting foreign and peripheral antigens to developing thymocytes – function is somewhat unclear

**6. The MOST critical concept to get about the thymus and T cell selection is how a T cell is ‘educated’ or selected based on T cell receptor and HLA interactions!!!!**

**Two Types of Selection (described in order below)**

***I. Positive Selection***

After a T cell has produced a rearranged T cell receptor (both alpha and beta chains) it is critical that the newly made TCR is tested to make sure it can properly interact with the host’s MHC (HLA in humans) molecules.

This is called ‘Positive Selection’ – the name refers to the signal that a T cell gets to survive only if the TCR can appropriately receive a signal from the MHC molecules expressed on thymic antigen presenting cells. This is sometimes called ‘death by neglect.’

Positive Selection will kill off 90% of all T cells that reach the DP stage – meaning they have rearranged productive TCRs. This is because the majority of randomly generated TCRs either cannot see the MHC molecules on the cTECs or because they bind the MHC molecules much too strongly and thus could attack the organism if they are allowed to mature.

In mice this is 45 million DP thymocytes that are killed by ‘neglect’ during positive selection every day.

***II. Negative Selection***

After Positive Selection a DP thymocyte begins to migrate to the medullary region and will lose expression of either CD4 or CD8 to become a single positive (SP4 or SP8) thymocyte.

In the Medulla the thymocyte will now be able to interact with medullary epithelial cells (mTEC).

mTEC express AIRE – which allows them to present peptides from all kinds of peripheral proteins (like Insulin) so that SP4 and SP8 thymocytes can be tested for reactivity against the organisms ‘SELF’ antigens.

Negative selection is thus the process of removing potentially ‘self’ or autoreactive T cells from the total pool.

**Together this process is referred to as ‘CENTRAL TOLERANCE’ – because it is the tolerance that occurs in the central organ for T cell development (Thymic Tolerance = CENTRAL TOLERANCE).**

**Thing to Consider! –** Medawar’s famous experiment demonstrated that you could induce tolerance to foreign HLA by injecting it into a fetal mouse at a specific stage of development. Why does it only work in the fetus? What is special about the fetus versus a young neonatal mouse or and adult mouse?

**7. Other Types of Cells Generated in the Thymus**

**Tolerogenic T cells (Regulatory T cells)**

Two types of Regulatory T cells (called Treg):

1) ‘Natural’ Treg – CD4+CD25++ cells produced in the thymus – typically 3-10% of SP4+ thymocytes. Also called ‘Thymic Treg’ - tTreg

Can sense and respond to the self-antigens presented during negative selection by AIRE+ mTECs or by thymic DCs – mechanisms unknown.

Function as dominant suppressors of T cell activation in the peripheral tissues

2) ‘Peripheral’ Treg – CD4+CD25++ cells that are generated in the peripheral tissues after activation by antigen presentinc cells (probably Dendritic cells)

Likely to be suppressive against commensal microorganisms – protect the host against inflammation that may come from conventional T cells responding to natural bacterial populations in the body (many in the gut).

Could be ‘pre-programmed’ to become Treg already – not well known.

-- BOTH natural and peripheral Treg require the transcription factor FOXP3 for their development, maintenance, and function. This is the MAIN way to identify these cells from other T cells.

**INVARIANT T cell Types**

**INVARIANT NK T cells (iNKT) –** express a specific alpha chain – V24-J18. Bind to a non-polymorphic MHC-like molecule called ‘CD1D’ which presents lipids and glycolipids rather than peptide antigens.

CD1d appears to be expressed by DP thymocytes so they mediate selection of these cells.

**Mucosal Associated INVARIANT T cells (MAIT) –** express a restricted set of TCR genes (V1-2-J12/20/33) that can recognize another non-classical MHC molecule called MR1 – MR1 presents compounds derived from bacterial vitamin B2 biosynthesis.

MR1 is expressed on thymocytes – so thymocytes serve to select these cells.

**Gamma/delta T cells –** non-conventional pathway of TCR rearrangement – most functions are not well known. A large class in humans (Vg9/Vd2) responds to microbial metabolites.

Unknown what ‘MHC’-like proteins are involved in g/d T cell activity – theorized that they respond to stress? Heat shock proteins?

**Extra Credit:**

***T cell receptor rearrangement actually occurs in several steps***

Step 1: Beta chain rearranges

Step 2: Beta chain is expressed paired with a gene called the ‘pre-TCRalpha’ chain.

* pTCRa is a gene that does not get rearranged – it is a decoy so that the beta chain can be tested before alpha chain rearrangement occurs
* If a good beta chain is made – it pairs with the pre-TCRa and is expressed on the surface. This allows the cell to survive and divide several times
* If a bad beta chain is made – another attempt can be made on the same (or on the other) allele until a good one is made. If not the cell dies.

Step 3: a real alpha chain undergoes rearrangement and can be paired with the selected beta chain

Step 4: The real TCR alpha/beta chain pair undergoes positive and then negative selection as described before

***T cell receptor gene loci are found on the maternal and paternal chromosomes meaning you have two copies of each.***

* This means you can actually make two functional TCRs potentially. (one beta chain and two possible TCR alpha chains)

T cells can express two functional, rearranged TCR alpha chains (one from the mom’s allele and one from the dad’s allele)

T cells CANNOT express two functional, rearranged TCR beta chains

* This is because the beta chain appears to undergo allelic exclusion so that one allele is silenced once a functional allele is selected during the first stage above

**Things to Consider!** – What might happen if you have a T cell that expresses two unique alpha chains and one beta chain?

Imagine you have a T cell with two alpha chains and one can sense a protein from the influenza virus (alpha1/beta TCR) and you get the Flu. This T cell is activated and undergoes clonal expansion to form highly inflammatory effector cells. What would be the possible consequences if this T cell clone could sense insulin protein with alpha2/beta receptor also expressed on its surface?

**Typical Exam Questions:**

**1) Describe the process of T cell selection in the thymus including 3 stages of thymocyte selection and two types of antigen presenting cells.**

**2) What process(es) ensures that mature T cells won’t get activated by the MHC found on host cells in the periphery?**

**3) How might you explain that type I diabetes could occur because of a defect in thymocyte selection? What stage is important to ensure that mature T cells don’t attack proteins found in different tissues (specific tissues like the pancreas, prostate, brain etc..)**

**4) What does ‘Immunological Tolerance’ mean – in general terms if your grandma asked what this is how would you describe it.**

**Here is a video summary of T cell development:**

[**https://www.youtube.com/watch?v=JeV-HuPq7CI**](https://www.youtube.com/watch?v=JeV-HuPq7CI)

**Here is a general overview from a famous Harvard Prof (Diane Mathis) on T cell tolerance including central (thymus) and peripheral (treg):**

**https://www.ibiology.org/immunology/t-cell-tolerance/**

**T CELL ACTIVATION**

**Three Main Stages**:

1. T cell must FIND the site where it’s cognate antigen is being presented

* Could be in the lymphoid tissues (Lymph Nodes, Spleen, Mucosal Lymphoid Tissues)
* Could be at the site of an infection (typically this would happen only in a secondary (memory) response

(why would a primary T cell response not be likely to happen in a non- lymphoid tissue)

1. T cell receives an activating signal from the antigen presenting cell that is expressing HLA with the cognate antigen/peptide

* Step 1) TCR binds HLA:peptide
* Step 2) many TCR molecules are recruited into a central synapse (called ‘immunological synapse’)
* Step 3) Activation of the T cell through signaling molecules associated with the TCR (CD3 proteins, CD4/CD8) causes the T cell to express surface proteins that activate receptors on the antigen presenting cell

1. The newly activated T cell begins to divide rapidly producing ‘clones’ with the same TCR which start to differentiate into various types of ‘effector’ T cells (more on this later) which can instruct other immune cells to help clear infections (helper T cells/CD4+) or kill infected cells (cytotoxic T cells/CD8)

(what about CD8+ T cells might make them best suited to kill infected cells? Hint: Has to do with how HLA molecules work)

**The early stages of T cell activation**

Naïve T cells express two important homing molecules on their surfaces – CCR7 and CD62 ligand (L-selectin).

These two migratory proteins help a naïve T cell to circulate through Secondary Lymphoid tissues (LNs, Spleen).

Note: primary lymphoid organs – thymus/bone marrow (where cells are made) – secondary LNs/Spleen – where cells are activated

A typical naïve T cell will move throughout the body in the blood entering and exiting lymph nodes – a ‘reactive’ lymph node where an ongoing inflammation is taking place will become especially good at attracting and retaining naïve T cells

When a dendritic cell in the tissues becomes activated by taking up a pathogen – it also upregulates CCR7 and lymph node homing markers and heads to a lymph node to present the antigen to a naïve T cell.

Only 100-10,000 naïve T cells in the entire body might be able to sense a specific viral peptide – so it takes time to bring the correct naïve T cells to the antigen presenting cell with its cognate antigen (remember 10^10+ naïve T cells!)

(keep in mind viruses have many proteins so you can have many independent cognate antigens activating diverse T cell clones – for example with yellow fever virus there are at least 5-6 peptide/HLA combinations that can give a detectable T cell response)

**The early stages of T cell activation**

Once a naïve T cell has found the antigen presenting cell with peptide it will stop moving and become gradually more fixed to the antigen presenting cell.

This happens rather quickly (seconds – minutes) but involves multiple steps including early activation by TCR stimulation, reorganization and upregulation of adhesion molecules to stabilize the T cell/APC engagement and generate an immunological synapse.

Signals delivered through the TCR (signal 1) causes the T cell to become activated and to upregulate CD40 ligand (CD40L) which engages its receptor (CD40) on the antigen presenting cell (signal 2).

This further activates the antigen presenting cell and causes release of pro-inflammatory cytokines (IL-12) which boost T cell activation and support T cell differentiation into inflammatory effectors

This is an oversimplification – different cytokines are going to have different effects on T cell differentiation (IL-12 is for T helper type 1 (Th1) responses)

Other types of responses (Th2, Th17, Treg) can also result from APC-T cell stimulation and depend on other signals

Finally clonal expansion occurs with individual naïve T cells dividing 10-20 times in a short period of time to generate hundreds of thousands or millions of clonal daughter cells.

This requires growth factors and metabolic energy – the most important growth factor to remember is Interleukin 2 (IL-2) which is produced primarily by activated CD4+ T cells.

**Checks and Balances on T cell Activation**

***Costimulation***

In addition to signal 2 – there are multiple other signaling complexes that exist during APC-T cell interactions.

The most well-described and one of the most important is interactions between a set of proteins called CD28 and CD80 (B7-1) or CD86 (B7-2). This is typically called ‘costimulation’ and it is important to strengthen the activation signals that the T cell is receiving through the TCR.

This pathway also serves a very important purpose for turning off a T cell – CD80 and CD86 can also engage a second receptor on T cells called CTLA-4.

CTLA-4 is a STOP signal and it will inhibit TCR signaling and activation.

CTLA-4 is upregulated by T cells in settings where the immune system perceives that an inappropriate response is occurring.

One such setting is cancer – where tumor cells will induce T cells to express CTLA-4 and thus the tumor can shut down T cell activation.

A Nobel Prize (2018) went to James Allison for demonstrating that antibodies which block CTLA-4 signaling (anti-CTLA4) can release tumor-reactive T cells from inhibition and allow them to attack cancer cells.

Another pathway involves PD-1 (programmed death 1) and PDL-1 and PDL-2. This can also be used to shut down immune responses and the second half of the Nobel prize went to discovery of antibodies to block this pathway which have similar effects to CTLA-4 blockade. The discoverer of PD-1 is Tasuku Honjo in Japan. Dr. Honjo ALSO discovered Activation induced Cytidine Deaminase (AID) which is a key protein involved in Antibody Class Switching and Somatic Hypermuation (B cell lectures from Lisa/Camilla).

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7349669/>

There is an excellent podcast on this linked below:

<https://www.embo.org/podcasts/from-cell-death-to-cancer-immunotherapy/>

This is a wonderful lecture for the Nobel prize from Jim Allison who is one of the greatest immunologists of all time.

<https://www.nobelprize.org/uploads/2018/10/allison-lecture.pdf>

(A major side-effect of CTLA4 or PD-1 blockade is autoimmunity – think about why that would happen? Also CTLA4 is for more aggressive than PD-1 blockade, what could be the reason for this?)

***Regulatory T cells***

Regulatory T cells are critical to maintain peripheral tolerance

If these cells are absent (because of mutations in the transcription factor FOXP3) then people develop terrible autoimmune disorders targeting many tissues (primarily intestine and endocrine tissues)

Exert dominant tolerance over other T cells by suppressing their activation or division through a variety of potential mechanisms.

1. Treg have high levels of the high affinity IL-2 receptor (CD25) so it is believed that they may take lots of IL-2 and prevent newly activated T cells from having proper access to this critical growth factor
2. Treg cells express CTLA-4 which has been suggested to have some sort of tolerizing properties for antigen presenting cells – so Treg may tell the APC to stop activating T cells

For costimulation CTLA-4 RECEIVES a negative signal from the APC – it appears it might actually deliver a negative signal too.

Here is a link to the paper I talked about in class on Tregs setting a threshold for T cell activation:

<https://www.science.org/doi/10.1126/science.1227049>

The previous youtube video I linked above in the central tolerance section also continues good information on Tregs.

**CD4 versus CD8 T Cell Activation**

**Fundamental Difference – MHC Class II (CD4) versus MHC Class I (CD8)**

This means that CD4+ T cells will be engaging ONLY with professional antigen presenting cells like dendritic cells and macrophages since ONLY these cells express MHC Class II.

CD8+ T cells engage MHC Class I meaning they can theoretically interact with any cell in the body (any nucleated cell – not erythrocytes)

For simplicity – let’s imagine that the typical immune response occurs in the following stages (updated now to distinguish CD4 and CD8 T cells):

1. A dendritic cell picks up antigen and brings it to the lymph node
2. A naïve CD4+ T cells senses the antigen and becomes activated
3. This leads to the CD4+ T cell upregulating CD40L which interacts with CD40 on the DC surface
4. This signal 2 ‘licenses’ the dendritic cell to become even more activated and to express inflammatory cytokines like IL-12.
5. CD80/CD86 that becomes upregulated on the dendritic cell engages with CD28 on the T cell amplifying activation signals
6. The highly activated dendritic cell recruits more CD4+ and CD8+ T cells which amplify the response
7. IL-12 produced by the dendritic cell causes CD4+ T cells receiving TCR engagement to become more inflammatory Th1 types of cells.
8. These Th1 CD4+ T cells start producing IL-2 which will also be important to promote proliferation of the activated CD8+ T cells.

This is a typical process that immunologist imagine during an early T cell activation response. It is obviously more complex and many more molecules are important but this is the general idea that you should be familiar with.

Because CD4+ T cells respond to MHC Class II this means they sense antigens that were taken up from the environment

CD8+ T cells respond to MHC Class I which means they respond to antigens that generated from intracellular proteins (proteins made in the cell – perhaps viral proteins?)

CD8+ T cells can also get activated by ‘cross presentation’ which is the process through which an environmental protein is expressed in the context of MHC Class I by antigen presenting cells – so that antigen presenting cells can present to CD8s even if they are not infected by a virus. Otherwise killed viral vaccines wouldn’t work!

**Types of CD4+ T cells:**

**Naïve CD4+ T cells** – CD4+ T cell produced from the thymus that has not seen antigen before. Defined by high expression of CCR7.

Th = T helper cell (nomenclature)

**Th1:** Make IL-2, IFN (interferon gamma), pro-inflammatory – typically associated with certain bacterial infections and viral infections. Very good at helping killer T cells (CD8+) and activating innate immune cells (macrophages) to be better killers (IL-12 induces them, T-bet is transcription factor they use)

**Th2:** Make IL-2, IL-4, IL-5, IL-13, pro-inflammatory – typically associated with allergies and worm infections (helminth). Help activate specific granulocytes of the innate immune system (eosinophils, basophils, mast cells) and aid in B cell activation and antibody production/class switching (IL-4 induces them, GATA3 is transcription factor they use)

**Th17:** Make IL-2, IL-17 (also IL-22 sometimes), pro-inflammatory – typically associated with specific types of bacterial infections. IL-17 and IL-22 both are involved in signaling to mucosal epithelial cells to help with their barrier functions. (IL-6 + TGF induces them, ROR is transcription factor they use)

**Th3/Tr1:** Make IL-10, immunosuppressive – thought to be a type of regulatory T cell probably important for mucosal (intestinal) immunity – not well characterized or understood. (IL-10 induces them, BLIMP-1 might regulate their function)

**Treg:** Maybe make IL-35 – not really cytokine makers. Immunosuppressive – thought to rely on CTLA-4 for their function. (TGF induces them, FOXP3 is the transcription factor they use)

**Tfh (follicular helper T cells):** . Home to B cell follicles by virtue of expressing CXCR5 (chemokine receptor for CXCL13). Play an important role in stimulating B cells to undergo clonal expansion and antigen-specific maturation of antibodies. May restrain the development of autoreactive B cells during affinity maturation. (IL-21 induces them, BCL6 is the transcription factor they use)

Different Th cells have different chemokine receptors (like CCR7) depending on where they are likely to go – for example Th1 cells have CXCR3 which is a receptor for chemokines produced in inflamed tissues. CXCR5 (the chemokine receptor which B cells use to home to follicles) defining Tfh is a great example of this.

Chemokine receptors are interesting ways to view different T cell types through – since the chemokine receptor expression pattern tells you where the T cell is going to go.

**Types of CD8+ T cells**

**Naïve CD8+ T cells** – CD8+ T cell produced from the thymus that has not seen antigen before. Defined by high expression of CCR7.

**Effector CD8+ T cells –** Make high levels of Granzymes (A, B, and H) and Perforin and secrete IFN. Actively kill cells which express cognate antigens.

DON’T express CCR7 – migrate to tissues with inflammation and NOT to lymph nodes

**Effector Memory CD8+ T cells –** ‘Poised’ to make granzymes/perforin/IFN but in a resting state – these cells arise from the activated effector pool AFTER an infection is over and circulate waiting to respond to secondary infections.

DON’T express CCR7 – likely circulate through tissues rather than in lymph nodes. Might be ‘first responder’ T cells whose job is to sense viral infections at the site of infection and recruit other cells quickly to shut down the response.

**Central Memory CD8+ T cells** – ‘Poised’ to help in secondary lymphoid tissues after an infection – may be more important to deliver signals to other cells rather than directly to kill. Or alternatively may produce effectors – not clear. Express CCR7 at intermediate levels. Don’t express high levels of Granzymes (A,B,H)/perforin/IFN. DO express Granzyme K – not clear what this does.

Effector and Effector Memory CD8+ T cells also make lots of chemokines (CCL3, CCL4, CCL5) which recruit activated T cells, macrophages, dendritic cells, NK cells etc… making them great ‘Alarmins’.

That means when a person is re-infected with a virus the effector memory cells may already be ready to send out chemokines to call inflammatory cells and amplify the immune response before the infection has time to get started.